# EXHIBIT D

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invited review

# Cardiovascular and renal control in NOS-deficient mouse models

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**Ortiz, Pablo A., and Jeffrey L. Garvin.** Cardiovascular and renal control in NOS-deficient mouse models. *Am J Physiol Regul Integr Comp Physiol* 284: R628–R638, 2003; 10.1152/ajpregu.00401.2002.—Nitric oxide (NO) plays an essential role in the maintenance of cardiovascular and renal homeostasis. Endogenous NO is produced by three different NO synthase (NOS) isoforms: endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS). To investigate which NOS is responsible for NO production in different tissues, NOS knockout (−/−) mice have been generated for the three isoforms. This review focuses on the regulation of cardiovascular and renal function in relation to blood pressure homeostasis in the different NOS−/− mice. Although regulation of vascular tone and cardiac function in eNOS−/− has been extensively studied, far less is known about renal function in these mice. eNOS−/− mice are hypertensive, but the mechanism responsible for their high blood pressure is still not clear. Less is known about cardiovascular and renal control in nNOS−/− mice, probably because their blood pressure is normal. Recent data suggest that nNOS plays important roles in cardiac function, renal homeostasis, and regulation of vascular tone under certain conditions, but these are only now beginning to be studied. Inasmuch as iNOS is absent from the cardiovascular system under physiological conditions, it may become important to blood pressure regulation only during pathological conditions related to inflammatory processes. However, iNOS is constitutively expressed in the kidney, where its function is largely unknown. Overall, the study of NOS knockout mice has been very useful and produced many answers, but it has also raised new questions. The appearance of compensatory mechanisms suggests the importance of the different isoforms to specific processes, but it also complicates interpretation of the data. In addition, deletion of a single gene may have physiologically significant effects in addition to those being studied. Thus the presence or absence of a specific phenotype may not reflect the most important physiological function of the absent gene.

endothelial nitric oxide synthase; neuronal nitric oxide synthase; inducible nitric oxide synthase; knockout mice; blood pressure

NITRIC OXIDE (NO) plays an important role in the maintenance of cardiovascular and renal homeostasis. In the cardiovascular system, it is involved in regulation of vascular tone (22), cardiac contractility (67), cell growth (76), vascular remodeling (49), and baroreflex function (7, 48, 111). In the kidney, NO regulates salt and fluid reabsorption (63, 89), hemodynamics (44), renin secretion (46), and tubuloglomerular feedback (TGF; 73, 106, 107). Most of these processes are important in both short- and long-term regulation of arterial blood pressure and have been extensively studied in the last 10 years.

Endogenous NO is enzymatically produced from conversion of the amino acid L-arginine to L-citrulline, a reaction catalyzed by the enzyme NO synthase (NOS). Three different NOS isoforms have been cloned and characterized: neuronal NOS (nNOS), endothelial NOS

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(eNOS), and inducible NOS (iNOS). The three isoforms are differentially expressed throughout the cardiovascular system and the kidney, and one or more isoforms can be expressed in the same cell type. To answer important questions regarding which isoforms produce the NO that regulates physiological processes, mice with disruption of each of the NOS genes have been generated. Studies of the different NOS knockout (−/−) mice have provided many answers but have also raised new questions regarding the role of the various NOS isoforms. Many reviews have concentrated on different pathophysiological aspects studied in NOS−/− mice (12, 25, 34, 35, 50, 78, 93). This review will focus on recent data concerning the regulation of cardiovascular and renal function in relation to blood pressure homeostasis in the different NOS−/− mice.

## eNOS−/−

*Blood pressure.* Given the location of e, n, and iNOS expression in the cardiovascular and renal systems, NO produced by each isoform has the potential to alter blood pressure. Most studies have shown that eNOS−/− mice have higher blood pressure than wild-type mice, although the magnitude of hypertension reported by different laboratories varies. Differences in systolic arterial pressure in conscious eNOS−/− compared with wild-type mice range from 20 (45) to 50 mmHg (85, 86), while differences in mean blood pressure in anesthetized mice range from 14 (5) to 37 mmHg (101). The varying magnitude of the hypertension observed in eNOS−/− may be due to the use of different methods for measuring blood pressure or the genetic backgrounds of the strains used. However, even with these differences, eNOS−/− mice were found to be hypertensive in all cases.

Arterial blood pressure is the product of cardiac output and total peripheral resistance. In turn, cardiac output depends on heart rate, cardiac contractility, and total extracellular fluid volume, which is maintained by the kidney; peripheral resistance is controlled by the tone of resistance vessels. It is frequently assumed that all hypertension in eNOS−/− is caused by the lack of endothelium-derived NO and the resulting increase in arterial tone and peripheral resistance. However, this simple explanation is not fully supported by the data, as discussed below.

*Vascular function.* The amount of eNOS expressed in the vascular endothelium, together with the data showing that NO is an important endothelium-derived relaxing factor, indicates that eNOS-derived NO is essential to regulation of vascular tone (22). Thus the hypertension observed in eNOS−/− can be partly attributed to increased vascular resistance caused by the lack of endothelial NO. Several investigators have studied the response of eNOS−/− arteries to vasodilator stimuli. It was first reported that aortic rings isolated from eNOS−/− do not relax in response to ACh (36). Lamping and Faraci (47) observed a complete lack of ACh-induced relaxation in carotid artery rings, with no difference between male and female mice. To verify that eNOS mediates ACh-induced relaxation in carotid arteries, Scotland et al. (80) transfected the endothelium of eNOS−/− mouse carotid arteries with an adenoviral vector carrying the gene for eNOS and in this way restored ACh-induced relaxation. These data indicate that *1*) in large vessels, such as the aorta or carotid artery, eNOS mediates ACh-induced vasodilatation and *2*) there is no compensatory vasodilator mechanism for ACh.

Despite the role of eNOS in major arteries, vascular peripheral resistance is primarily controlled by small resistance arterioles. In contrast to large arteries, other endothelium-derived vasodilators have been shown to compensate for the lack of eNOS in resistance arteries. Myogenic responses and ACh-induced relaxation are preserved in mesenteric arteries of eNOS−/−. These responses could be prevented by $K^+$ channel blockers in eNOS−/− but not in wild-type mice, suggesting that they are mediated by an endothelium-derived hyperpolarizing factor (EDHF) (18, 79). Sun et al. (91) reported that in gracilis muscle arterioles of male eNOS−/−, flow-induced dilatation was completely blocked by indomethacin, whereas this drug only inhibited 50% of the response in wild-type mice. Interestingly, the same group reported that in female eNOS−/−, a different vasodilator mechanism compensates for the lack of eNOS. In females, flow-induced vasodilatation was not prevented by indomethacin but was completely blocked by a $Ca^{2+}$-activated $K^+$ channel blocker or a cytochrome P450 inhibitor (31). A gender difference in the relative contribution of NO to endothelium-dependent vasodilatation has been reported in wild-type mice and in rats (31, 32, 55, 91). Thus a different compensatory mechanism could be activated in the absence of eNOS in male or female mice. However, the precise explanation for these differences is still not known.

On the basis of these data, it is possible to conclude that eNOS-derived NO is an important mediator of vasodilator stimuli that affect vascular tone in most arteries studied. In large arteries the lack of endothelial NO in eNOS−/− impairs the relaxant effect of vasodilators, and this is apparently not compensated for by other endothelial vasodilators. In resistance arteries, the lack of eNOS is sometimes compensated for by EDHF, a cyclooxygenase product, or by nNOS-derived NO, as in the brain pial arterioles and the coronary microcirculation (33, 56) (Fig. 1). It is important to note that in some cases such as the pulmonary circulation, the lack of eNOS is not compensated for at all (20). Thus most data indicate that the mechanisms that compensate for the lack of eNOS-derived NO are specific to different vascular beds, and one or more mechanisms may be activated to regulate arterial tone in the absence of eNOS. Although the absence of eNOS appears to be compensated for in most resistance arteries, eNOS−/− mice exhibit higher blood pressure and it is still not known whether they have increased total peripheral resistance. To us, this raises the questions of how much of the hypertension observed in eNOS−/− is caused by an increase in vascular tone



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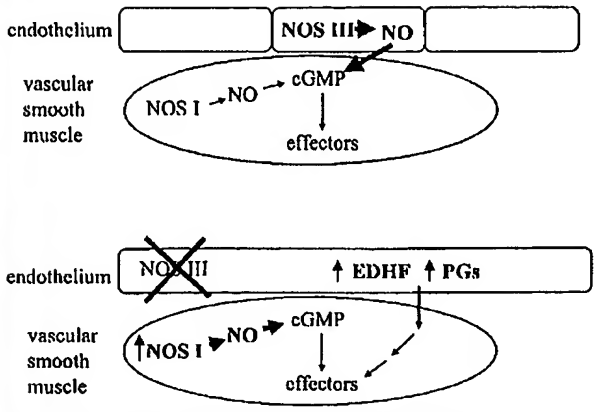


Fig. 1. Vasodilator mechanisms that compensate for the lack of endothelium-derived nitric oxide (NO) in resistance arterioles of endothelial NO synthase knockout (eNOS−/−) mice. EDHF, endothelium-derived hyperpolarizing factor.

and whether other mechanisms such as increased cardiac output and increased salt and fluid absorption by the kidney may be involved in the hypertension exhibited by these mice.

*Renal function.* Extracellular fluid volume is regulated by the kidney, where eNOS is expressed in the endothelium of the renal vasculature, including the afferent and efferent arterioles and vasa recta. eNOS is also present in proximal tubules, thick ascending limbs, and collecting ducts (44, 96). However, despite the important role of NO in the regulation of various physiological processes in the kidney, little is known about the contribution of the three NOS isoforms to these mechanisms.

NO is known to be involved in the regulation of renin secretion. However, the data are still contradictory, with some reports concluding that endothelial NO stimulates renin secretion, whereas others found the opposite (46). In eNOS−/−, a decrease in total kidney renin mRNA was observed, whereas plasma renin levels were elevated by 50% compared with wild-type mice despite the high blood pressure (81). These data suggest that eNOS-derived NO tonically inhibits renin release and that regulation of renin release by renal perfusion pressure is impaired because renin levels would be low due to the high blood pressure. Wagner et al. (101) found a decrease in renin mRNA levels in total kidney homogenates but also reported lower levels of renin activity in the eNOS−/− kidney. Although this latter result contradicts the increase in plasma renin observed by others, total renin activity does not necessarily reflect plasma levels or rate of renin secretion. In a recent study, Beierwaltes et al. (5) found no difference in plasma renin content between eNOS−/− and wild-type mice. They also found that in anesthetized mice, renin secretion in response to reduced perfusion pressure was normal in eNOS−/−, whereas acute inhibition of NOS completely prevented pressure-dependent renin release in wild-type mice. The authors concluded that pressure-dependent renin release is completely compensated for in eNOS−/−. Inasmuch as NO derived from macula densa nNOS regulates renin secretion, it is possible that this pathway is upregulated in eNOS−/−. It is important to note that plasma renin levels have been found to be either equal or increased in eNOS−/− in the context of increased blood pressure (5, 81), suggesting that this mechanism may contribute to hypertension. The precise role of NO in the regulation of renin secretion is still unknown, as are the molecular mechanisms by which NO acts in renin-secreting cells.

Previous in vivo and in vitro data support a role for endothelium-derived NO in the regulation of renal vascular tone (44). In eNOS−/−, basal renal perfusion pressure was increased, but renal blood flow was similar to wild-type mice (5). Acute infusion of the NOS inhibitor $N^G$-nitro-L-arginine methyl ester (L-NAME) decreased renal blood flow in wild-type mice but had no effect in eNOS−/−. These data suggest that in the renal vasculature compensatory mechanisms are activated in the absence of eNOS to maintain normal renal blood flow. However, the mechanism by which renal blood flow is maintained is not known. In rabbit afferent arterioles, NOS inhibition reduced basal arteriolar diameter (38, 43). In contrast, Patzak et al. (66) reported that in isolated perfused afferent arterioles from wild-type mice, neither L-arginine nor L-NAME changed basal arteriole diameter, suggesting that basal NO activity is negligible in this preparation. However, angiotensin II-induced contractions were greatly enhanced in afferent arterioles of eNOS−/−, suggesting that eNOS-derived NO decreases the constrictor response to angiotensin II. Although NO has been shown to modulate the vasoconstrictor response to other hormones, the role of eNOS-derived NO in these responses has not been studied to our knowledge.

NO is an important regulator of the renal medullary microcirculation (53, 65). Medullary infusion of the nonspecific NOS inhibitor L-NAME decreases medullary blood flow and increases sodium retention and blood pressure (54). Conflicting results have been published regarding a role for nNOS in this process. It was first shown that infusion of anti-sense oligonucleotides for nNOS into the medullary interstitium caused salt-sensitive hypertension in rats (51, 52), suggesting a role for nNOS in blood flow regulation. However, infusion of selective nNOS inhibitors into the renal medulla did not affect medullary blood flow, although it decreased NO levels (41), suggesting that nNOS does not play a role in medullary blood flow regulation. Thus the particular NOS isoform responsible for regulation of medullary blood flow or the cell type where the NO that affects blood flow is produced is unknown. In the renal medulla, NO could be produced by the thick ascending limb (62), descending vasa recta (75), or medullary collecting duct (11, 57). Despite the important role of NO in the medullary microcirculation, NOS knockout mice have not yet been used to study this physiological process.

NO has been shown to inhibit NaCl and fluid reabsorption along the nephron and promote renal sodium



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and water excretion; however, very little is known about the role of endogenous NO and the contribution of the different NOS isoforms to this process (63). We reported that in the rat thick ascending limb, L-arginine stimulates endogenous NO production and decreases NaCl and $NaHCO_3$ reabsorption (60–62, 70). To investigate which NOS isoform mediates this response, we studied the effect of L-arginine on thick ascending limbs from eNOS−/−, iNOS−/−, and nNOS−/− mice. We found that in thick limbs from wild-type, nNOS−/−, and iNOS−/− mice, L-arginine inhibited NaCl absorption, whereas it had no effect in eNOS−/−. A NO donor was able to inhibit NaCl transport in eNOS−/−, indicating that the second messenger cascade for NO was intact (69). These data suggest that eNOS-derived NO inhibits NaCl absorption in this nephron segment and that the lack of eNOS is not compensated for by other NOS isoforms.

Although the proximal tubule and collecting duct also express eNOS, little is known about basal or stimulated reabsorption rates in these tubular segments from eNOS−/−. In proximal tubules microperfused in vivo, Wang (103) reported no differences in basal fluid and bicarbonate reabsorption rates in eNOS−/− compared with wild-type mice. In contrast, Adler et al. (1) reported that basal oxygen consumption in cortical renal slices was higher in eNOS−/− than in wild-type mice. The renal cortex is composed mostly of proximal tubules and a minor fraction of vascular cells, distal tubules, and cortical thick ascending limbs. Because the basal metabolic rate of epithelial cells is much higher than that of vascular cells, and because NO has been shown to inhibit proximal tubule sodium reabsorption (63), the higher rate of oxygen consumption found in eNOS−/− is likely due to increased basal rates of sodium reabsorption by the proximal tubule, caused by the lack of eNOS-derived NO. However, the precise role of eNOS in the proximal tubule is currently unknown. Thus it is possible that a lack of eNOS-derived NO may chronically increase reabsorption of NaCl by the nephron and increase TGF responses, contributing to the hypertension observed in eNOS−/− (Fig. 2).

*Cardiac function.* Elimination of NO produced by eNOS in the heart may also enhance cardiac output and contractility. Recently, a large number of publications have centered on the role of NO in regulation of cardiac function. In the heart, eNOS is expressed not only in the endothelium of the coronary vessels but also in cardiac myocytes. In addition to eNOS, cardiac myocytes have also been shown to express nNOS in the mitochondria and sarcoplasmic reticulum (42, 110), suggesting that NO plays an important role in myocyte physiology.

Most investigators have reported that the basal heart rate in conscious eNOS−/− mice is significantly lower than in wild-type mice (23, 45, 81, 85, 110). However, others have reported that heart rates measured in anesthetized animals were no different between eNOS−/− and wild-type mice (27, 36). An acute increase in blood pressure decreases the heart rate via a baroreflex mechanism. However, during long-term increases in blood pressure, the baroreceptor resets to the new pressure and the heart rate returns to baseline. In eNOS−/−, the increase in blood pressure is chronic and thus the heart rate would be expected to be normal. Although there is still no explanation for the decreased heart rate observed in conscious eNOS−/−, it is possible that eNOS-derived NO can affect baroreflex resetting or be involved in establishing the baroreceptor setpoint (30, 48, 84). In addition, the fact that the heart rate is not different in anesthetized mice may reflect the loss of baroreflex influence caused by the

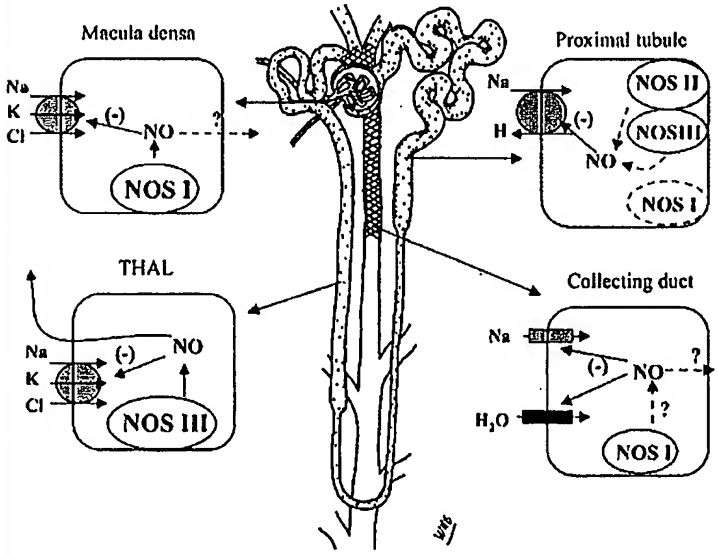


Fig. 2. Role of the different NOS isoforms along the nephron. Dashed line indicates no direct experimental evidence.



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anesthesia. Overall, it is still not clear why eNOS−/− have a lower heart rate than wild-type mice.

In eNOS−/− mice, basal parameters of cardiac function in vivo appear to be normal. Assessment of cardiac function by echocardiography showed no significant differences in left ventricular shortening fraction, ejection fraction, and cardiac output between eNOS−/− and wild-type mice (110). Basal systolic contractility, reflected by the maximum rate of pressure development ($dP/dt_{max}$), was no different from wild-type mice as measured with an intraventricular pressure catheter (27). An increase in left ventricular mass and posterior wall thickness together with an increase in myocyte size indicative of cardiac hypertrophy have been observed in eNOS−/− (110). Although these changes are most likely due to hypertension, a role for NO in cardiac myocyte growth cannot be ruled out.

Basal cardiac contractility was found to be similar between eNOS−/− and wild-type mice. However, data obtained from perfused whole heart (Langendorff) preparations or in vivo suggest that during β-adrenergic stimulation cardiac contractility is influenced by eNOS-derived NO. Gyurko et al. (27) found that in an isolated perfused whole heart preparation, isoproterenol-stimulated cardiac contractility was enhanced in hearts from eNOS−/−, whereas β-adrenergic receptor density was no different between eNOS−/− and wild-type mice. In vivo assessment of cardiac function showed that isoproterenol-stimulated cardiac contractility was enhanced in eNOS−/− compared with wild-type mice. The effect of isoproterenol in eNOS−/− was similar to the response observed in wild-type mice treated with the NOS inhibitor L-NNA, supporting a role for eNOS in mediating this effect (27). Another group of investigators also reported that isoproterenol-stimulated cardiac contractility was enhanced in hearts from eNOS−/− mice (100). These data indicate that in the intact animal, eNOS-derived NO modulates the systolic response to β-adrenergic stimulation.

The mechanism by which eNOS modulates β-adrenergic-stimulated cardiac contractility appears to involve activation of $\beta_3$-adrenergic receptors. It has been shown that in $\beta_3$-adrenergic receptor knockout mice ($\beta_3$−/−), the contractile response to isoproterenol is enhanced to the same extent as in eNOS−/− (100). In addition, NOS inhibitors enhance the β-adrenergic inotropic response in wild-type mice but not in $\beta_3$−/−. These data suggest that binding of isoproterenol to the $\beta_1$- and $\beta_2$-adrenergic receptors stimulates cardiac contractility, whereas simultaneous binding to the $\beta_3$ receptor modulates the positive inotropic effect by activating eNOS. A recent report confirms the latter hypothesis by showing that a selective $\beta_3$-receptor agonist decreased intracellular calcium and sarcomere length in wild-type cardiac myocytes but failed to produce this effect in myocytes from eNOS−/− (4). However, the mechanism for the increase in intracellular calcium observed in eNOS−/− mice remains unclear.

Whereas most data support a role for eNOS in decreasing β-adrenergic effects on contractility, in vitro studies using isolated myocytes are more controversial. Han et al. (28) first reported that in isolated cardiac myocytes from eNOS−/−, the muscarinic agonist carbachol failed to reverse isoproterenol-stimulated contractions. These authors showed that carbachol failed to decrease isoproterenol-stimulated L-type $Ca^{2+}$ channel activation in eNOS−/− mice, whereas it completely abolished the effect of isoproterenol in myocytes from wild-type mice. Although these data are consistent with eNOS-derived NO blunting β-adrenergic stimulation of contractility, others have failed to reproduce these results. Vandecasteele et al. (99) found no difference in β-adrenergic-induced contractility of isolated papillary muscles between eNOS−/− and wild-type mice. They also reported that carbachol blocks isoproterenol-stimulated L-type $Ca^{2+}$ channel currents to the same extent in isolated eNOS−/− and wild-type myocytes. In agreement with the last report, two other groups of investigators found no differences in isoproterenol-stimulated L-type $Ca^{2+}$ channel currents or the inhibitory effect of muscarinic receptor agonists between isolated eNOS−/− and wild-type myocytes (6, 24). Consistent with a role for eNOS in decreasing the β-adrenergic increase in contractility, Barouch et al. (4) found that the isoproterenol-stimulated increase in intracellular calcium was enhanced in myocytes from eNOS−/−. Contractility data obtained from isolated myocytes are still unresolved. The different results observed in isolated cell preparations may be attributable to blockade or activation of second messenger cascades caused by physical disruption of the tissue or the different protocols used to obtain it. Although the data suggest that eNOS-derived NO affects intracellular calcium balance, the mechanism involved is still unclear.

In summary, in vivo and in vitro data indicate that, under basal conditions, cardiac function is normal in eNOS−/−, suggesting that the lack of eNOS is compensated for by other mechanisms or that eNOS only plays a minor role in basal cardiac function. However, in vivo data indicate that when sympathetic output and adrenergic discharge are increased, eNOS mediates the negative inotropic effect caused by stimulation of the $\beta_3$-adrenergic receptor. Thus eNOS-derived NO appears to be an important physiological modulator of cardiac contractility.

Finally, genetic deletion of eNOS may disrupt the function of other important regulators of blood pressure by affecting central nervous system activity. For example, Stauss et al. (85, 87) found that blood pressure is more variable in eNOS−/− compared with wild-type mice, suggesting that baroreflex responses are blunted in the former. Still, little is known about other central nervous system effects of deleting eNOS, in particular regarding blood pressure control.

Studies in eNOS−/− mice have clearly demonstrated that NO produced by eNOS plays an important role in the regulation of blood pressure. Although one might expect that eliminating eNOS from the vasculature would play a predominant role in the hypertension seen in eNOS−/− mice, other vasodilators partially compensate for the loss of eNOS in resistance



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vessels. Thus the hypertension observed in these mice can also be attributed to the lack of eNOS-derived NO in the kidney and heart.

nNOS−/−

*Blood pressure.* In contrast to the findings observed in eNOS−/−, blood pressure in nNOS−/− mice has generally been shown to be similar to that of wild-type controls (4, 40, 59, 98). These data suggest that genetic deletion of nNOS is compensated for, in terms of blood pressure regulation. Alternatively, the hypotensive actions of nNOS may be counterbalanced by its hypertensive effects. Acute administration of the nNOS inhibitor 7-nitroindazole (7-NI) to eNOS−/− mice significantly reduced blood pressure (45), suggesting that nNOS contributes to their hypertension. However, Barouch et al. (4) reported that systolic blood pressure in mice deficient in both eNOS and nNOS (e-nNOS−/−) was higher than in wild-type mice and similar to eNOS−/−. Although nNOS may be antihypertensive in some cases (74, 106), in others it appears to be prohypertensive (4, 45). Thus the role of nNOS in the global regulation of blood pressure is still not well defined, and more work is needed in this area.

*Vascular function.* The data showing that blood pressure in nNOS−/− is similar to that of wild-type mice suggest that nNOS does not play an important role in the regulation of basal vascular tone. However, it has been shown that nNOS is expressed in vascular smooth muscle cells (10, 33) and also in cardiac myocytes (110). Therefore, while vascular tone is regulated by eNOS under basal conditions, it is possible that when eNOS-dependent vasodilatation is impaired, nNOS-derived NO could modulate vascular tone. For example, in brain pial arterioles of eNOS−/−, ACh-induced dilatation was found to be reduced by only 25% compared with wild-type controls. In the presence of the nNOS inhibitor 7-NI, ACh-induced dilatation was reduced by 50% in eNOS−/− but normal in wild-type mice, suggesting that nNOS-derived NO compensates for the lack of eNOS (56). This is supported by Huang's study (33), showing that in isolated perfused coronary arterioles of eNOS−/−, which exhibited normal flow-induced dilatation, 7-NI blunted flow-induced dilatation by 40% but had no effect in wild-type arterioles. In addition, these authors found upregulation of nNOS expression in the endothelium and smooth muscle of coronary arteries. Brandes et al. (9) observed increased sensitivity of soluble guanylate cyclase in aortic rings of eNOS−/−. These data suggest that despite the small amount of nNOS in blood vessels, low levels of nNOS-derived NO could compensate for the lack of eNOS in these mice. To date, compensation by nNOS has only been evident in eNOS−/− arterioles, but it could also be important in other conditions where endothelium-dependent vasodilatation is impaired, such as hypercholesterolemia (8, 90) and diabetes (16).

*Renal function.* In the kidney, nNOS is expressed in macula densa cells, collecting ducts (77, 105, 107), and in thick ascending limbs (Garvin, unpublished observations). nNOS expression is significantly higher in the macula densa compared with other tubular cells, suggesting an important role for this isoform in modulating the function of the macula densa and juxtaglomerular apparatus (106). In fact, studies using pharmacological inhibitors of NOS have shown that nNOS-derived NO produced in the macula densa blunts TGF responses in rats, rabbits, and mice (74, 95, 98). In wild-type mice, inhibition of macula densa nNOS with 7-NI increases the magnitude of the TGF response, seen as greater constriction of the afferent arteriole (74). Vallon et al. (98) studied TGF responses in nNOS−/− in vivo by monitoring changes in proximal stop-flow pressure while increasing luminal perfusion rates to the distal nephron. They found no difference in TGF responses between nNOS−/− and wild-type mice; however, blocking NOS with L-NNA increased TGF in wild-type mice but had no effect in nNOS−/−. In agreement with this report, we found no difference in TGF responses between nNOS−/− and wild-type mice. However, 7-NI potentiated TGF responses in wild-type mice, whereas it did not affect TGF in nNOS−/− (74). Taken together, these data suggest that in wild-type mice, nNOS-derived NO produced in the macula densa blunts TGF. However, chronic deletion of nNOS is compensated for by some mechanism that helps maintain glomerular hemodynamics. Because we have shown that NO produced by the thick ascending limb (presumably by eNOS) can also blunt TGF responses (102), it could be that this mechanism is upregulated in nNOS−/− to maintain normal TGF.

The precise mechanism by which nNOS-derived NO attenuates the TGF response is currently unknown. The published data allow for speculation on some possible mechanisms that could mediate the effects of NO in TGF. Data from our laboratory have shown that inhibition of soluble guanylate cyclase or protein kinase G in the macula densa, but not in the afferent arteriole, blunts TGF similarly to inhibition of nNOS (73). We have also shown that in the thick ascending limb NO decreases the activity of the apical Na-K-2Cl cotransporter, which is also located in the macula densa and is known to initiate TGF (64). Thus one possible mechanism is that NO acts in an autocrine manner in the macula densa, blunting TGF by tonically inhibiting NaCl entry via the Na-K-2Cl cotransporter. Other mechanisms may involve the inhibition of 5′-ectonucleotidase by NO. Adenosine is a mediator of the TGF response (68, 71, 92, 94), and it could be released by the macula densa or produced in the interstitium by enzymatic degradation of ATP, ADP, and AMP by 5′-ectonucleotidase. Because NO has been shown to inhibit this enzyme (82), it is possible that NO blunts TGF by tonically inhibiting it and thus decreasing adenosine levels. Another possibility is that NO produced in the macula densa diffuses through the interstitial space and activates soluble guanylate cyclase in smooth muscle cells, increasing cGMP levels and dilating the afferent arteriole. Finally, new mechanisms could be hypothesized in view of recent data



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showing that intact extraglomerular mesangial cells and their gap junctions are necessary for TGF (72).

nNOS has also been shown to be expressed in the collecting duct (77, 105), though at lower levels than in macula densa cells. Although NO has been found to inhibit sodium and fluid transport in this segment (63), to our knowledge there are no studies regarding the contribution of nNOS to this effect. There is still no evidence for functional expression of nNOS in the proximal tubule, and the effects of NO on proximal tubule transport remain controversial. In vivo data have shown that NO can inhibit and stimulate sodium and fluid reabsorption, while most in vitro data are consistent with NO inhibiting transport in this segment (63). In support of an inhibitory effect of endogenous NO on proximal tubule transport, Vallon et al. (98) observed that in vivo microperfused proximal tubules of nNOS−/− exhibited higher fluid and chloride absorption rates compared with proximal tubules of normal mice, suggesting that endogenously produced NO inhibits proximal tubule transport. In contrast to the study of Vallon et al. (98), Wang et al. (104) reported that nNOS knockout mice exhibited lower fluid and bicarbonate absorption rates than proximal tubules from wild-type mice, suggesting that NO produced by nNOS stimulates rather than inhibits transport in the proximal tubule.

The explanation for the disparate results regarding the role of nNOS-derived NO in proximal tubule transport is unclear. However, in nNOS−/− mice, nNOS is genetically deleted from all tissues, not just the proximal tubule. Thus the difference between wild-type and nNOS−/− may be due to the effect of deleting nNOS from other organs or a change in the control of neural innervation of the proximal tubule. In fact, it has been shown that the effects of NOS inhibition in proximal tubule transport are modulated by different degrees of neural activity (63, 108).

*Cardiac function.* In the heart, nNOS is found in the sarcoplasmic reticulum (109) and mitochondria of cardiac myocytes (42), cholinergic and nonadrenergic/noncholinergic nerve terminals, and in sympathetic nerve terminals, where it has been postulated to play a role in catecholamine release and reuptake (14, 15, 40). Although blood pressure is normal in nNOS−/−, studies have shown that this NOS isoform is important for maintenance of normal cardiac function. Basal heart rates have been found to be slightly increased in nNOS−/− (14, 40). It has also been reported that heart rate variability is decreased in nNOS−/−, suggesting that the increased heart rate may be due to reduced parasympathetic tone (40). In addition, atropine, a muscarinic antagonist, increased the heart rate in wild-type mice but had no effect in nNOS−/−, suggesting that muscarinic tone was already blunted (40). In agreement with nNOS controlling heart rate, Choate et al. (14) reported that vagal nerve stimulation caused a much slower decrease in nNOS−/− heart rate compared with wild-type mice, but the magnitude of the response was similar in both strains. The heart rate decrease caused by a muscarinic agonist was similar in both strains. These data suggest that vagal control of bradycardia is modulated by nNOS-derived NO via a presynaptic mechanism. The exact mechanism by which nNOS-derived NO modulates parasympathetic tone has not been fully studied to our knowledge.

The presence of nNOS in the sarcoplasmic reticulum of cardiac myocytes has led to the hypothesis that nNOS-derived NO modulates calcium fluxes and myocardial contractility (110). Data presented in two recent studies support this hypothesis. Ashley et al. (3) found that in isolated cardiac myocytes the percentage of cell shortening (an indicator of contractility) was enhanced in nNOS−/− myocytes during electrical stimulation. In addition, the contractile response to isoproterenol was enhanced in nNOS−/− myocytes. Similarly, Barouch et al. (4) found that in vivo basal cardiac contractility was enhanced in nNOS−/− as shown by increased $dP/dt_{max}$. However, when they studied the β-adrenergic response, they found decreased isoproterenol-stimulated contractility in nNOS−/− compared with wild-type mice. These authors also reported that isoproterenol-stimulated increases in intracellular calcium and contractility in isolated cardiac myocytes were almost completely abolished in nNOS−/−. These data suggest that nNOS-derived NO increases intracellular calcium and cardiac contractility, whereas eNOS-derived NO has the opposite effect. Interestingly, in double e-nNOS−/− mice basal cardiac contractility in vivo was even higher than in nNOS−/−, eNOS−/−, and wild-type hearts and isoproterenol-stimulated contractility was almost normal (4). Overall, the data suggest that under basal conditions both eNOS and nNOS decrease contractility; however, during β-adrenergic stimulation, eNOS decreases contractility while nNOS increases it (Fig. 3). As with other intracellular signaling molecules (e.g., cAMP), the effect of the second messenger is regulated by strictly controlling the site of production and the intracellular location of the target protein complexes (21, 39, 83). For NO signaling, these data suggest a new level of regulation that has not yet been studied.

### iNOS−/−

*Blood pressure.* The gene locus coding for iNOS has been shown to cosegregate with blood pressure in the Dahl salt-sensitive rat (17). Whereas in most tissues iNOS is only induced by proinflammatory factors, it is expressed constitutively in the renal medulla (44). Despite the implication that iNOS may be involved in the development of salt-sensitive hypertension, there have been very few studies testing this hypothesis or questioning the role of iNOS in blood pressure regulation. Recently, Ihrig et al. (37) reported that basal systolic blood pressure was elevated by 10 mmHg in iNOS−/− at 3 mo of age, but was no different from wild-type mice at 9 or 12 mo. Feeding iNOS−/− a high-salt diet for 8 wk did not increase blood pressure further by 3 mo and had no effect at 9 to 12 mo. Interestingly, and similar to reports in eNOS−/− mice, iNOS−/− had higher plasma cholesterol levels than the wild type (19, 37). Ullrich et al. (97) also observed no differences in mean



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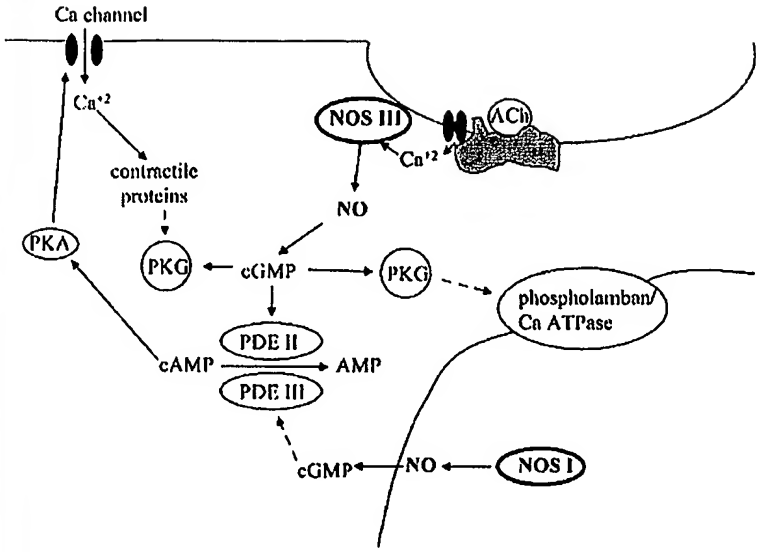


Fig. 3. Potential role of the different NOS isoforms in cardiac myocytes. PKG, protein kinase G; PDE II, cGMP-stimulated phosphodiesterase; PDE III, cGMP-inhibited phosphodiesterase; PKA, protein kinase A.

arterial pressure in 2- to 5-mo old iNOS−/−. We could find no other studies regarding blood pressure regulation in iNOS−/− under physiological conditions, nor of its relationship to age, salt sensitivity, and other vasoactive hormones. Thus more work in this area is needed.

*Vascular function.* iNOS expression has not been found in the vasculature under physiological conditions and thus is not likely to play a role in basal regulation of vascular tone. Only after its induction by inflammatory factors such as lipopolysaccharides, tumor necrosis factor, or interleukin-1 has iNOS-derived NO been shown to affect vascular tone (13, 88, 97). Given that iNOS induction appears to occur only under pathological conditions (i.e., septic shock) and there are few studies on its role in vascular tone regulation in iNOS−/− mice, it will not be discussed further here.

*Renal function.* In contrast to findings in other organs, iNOS is constitutively expressed in the renal medulla and proximal tubule (2, 44). However, the role of iNOS in the regulation of nephron transport has not been studied extensively. It was first reported that in proximal tubules stimulated with lipopolysaccharides, L-arginine decreased $Na^+$-$K^+$-ATPase activity, consistent with data showing that exogenous NO inhibits both apical $Na^+$ entry and basolateral $Na^+$ pump activity in this segment (26). In contrast to previous results, Wang (103) recently reported that in proximal tubules of iNOS−/− perfused in vivo, basal fluid and bicarbonate reabsorption were lower than in wild-type mice. They concluded that under basal conditions NO produced by iNOS in the proximal tubule stimulates solute and fluid reabsorption. There is currently no explanation for these disparate results, and more data are needed to resolve this issue.

*Cardiac function.* We know of no data regarding iNOS expression in mouse hearts under basal conditions. Ullrich et al. (97) reported that iNOS−/− mice have a heart rate similar to that of wild-type mice as well as normal blood pressure. It has been proposed that iNOS induction in the heart during chronic inflammation may lead to heart failure and other deleterious effects. However, it is still not defined whether iNOS induction is important to the development of heart failure or whether it is a consequence of the inflammatory response. Conflicting results have been obtained from experiments in which iNOS was overexpressed in cardiac myocytes. Heger et al. (29) found that iNOS overexpression caused a small decrease in heart rate and cardiac output but no other abnormalities in cardiac function, histology or anatomy. However, Mungrue et al. (58) found increased ventricular size, abnormal conduction, and increased mortality in these mice. Although the different results may be due to different levels of iNOS expression in these mice, the precise role of iNOS in the heart is still unclear.

## CONCLUDING REMARKS

Genetic deletion of the various NOS isoforms has greatly aided our understanding of how NO and the three NOS isoforms regulate blood pressure and cardiovascular/renal function. However, many questions remain that cannot be answered with these models. Further clarification will require the development of inducible tissue-specific knockout of e, i, and nNOS. Additionally, the completion of the Human Genome Project has fundamentally changed the way we describe a gene and its function. It is now apparent that the concept of "one gene, one protein" was naive. Genetic deletion of a single gene may have physiologically significant effects in addition to, or more important than, those being studied. Thus the set of parameters we choose to study in knockout mice may not in fact reflect the most important physiological function of the absent gene.



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## REFERENCES

1. **Adler S, Huang H, Loke KE, Xu X, Tada H, Laumas A, and Hintze TH.** Endothelial nitric oxide synthase plays an essential role in regulation of renal oxygen consumption by NO. *Am J Physiol Renal Physiol* 280: F838–F843, 2001.
2. **Ahn KY, Mohaupt MG, Madsen KM, and Kone BC.** In situ hybridization localization of mRNA encoding inducible nitric oxide synthase in rat kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F748–F757, 1994.
3. **Ashley EA, Sears CE, Bryant SM, Watkins HC, and Casadei B.** Cardiac nitric oxide synthase 1 regulates basal and beta-adrenergic contractility in murine ventricular myocytes. *Circulation* 105: 3011–3016, 2002.
4. **Barouch LA, Harrison RW, Skaf MW, Rosas GO, Cappola TP, Kobeissi ZA, Hobai IA, Lemmon CA, Burnett AL, O'Rourke B, Rodriguez ER, Huang PL, Lima JA, Berkowitz DE, and Hare JM.** Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. *Nature* 416: 337–339, 2002.
5. **Beierwaltes WH, Potter DL, and Shesely EG.** Renal baroreceptor-stimulated renin in the eNOS knockout mouse. *Am J Physiol Renal Physiol* 282: F59–F64, 2002.
6. **Belevych AE and Harvey RD.** Muscarinic inhibitory and stimulatory regulation of the L-type $Ca^{2+}$ current is not altered in cardiac ventricular myocytes from mice lacking endothelial nitric oxide synthase. *J. Physiol* 528: 279–289, 2000.
7. **Bielefeldt K, Whiteis CA, Chapleau MW, and Abboud FM.** Nitric oxide enhances slow inactivation of voltage-dependent sodium currents in rat nodose neurons. *Neurosci Lett* 271: 159–162, 1999.
8. **Blair A, Shaul PW, Yuhanna IS, Conrad PA, and Smart EJ.** Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmalemmal caveolae and impairs eNOS activation. *J Biol Chem* 274: 32512–32519, 1999.
9. **Brandes RP, Kim D, Schmitz-Winnenthal FH, Amidi M, Godecke A, Mulsch A, and Busse R.** Increased nitrovasodilator sensitivity in endothelial nitric oxide synthase knockout mice: role of soluble guanylyl cyclase. *Hypertension* 35: 231–236, 2000.
10. **Brophy CM, Knoepp L, Xin J, and Pollock JS.** Functional expression of NOS 1 in vascular smooth muscle. *Am J Physiol Heart Circ Physiol* 278: H991–H997, 2000.
11. **Cai Z, Xin J, Pollock DM, and Pollock JS.** Shear stress-mediated NO production in inner medullary collecting duct cells. *Am J Physiol Renal Physiol* 279: F270–F274, 2000.
12. **Chan PH, Epstein CJ, Li Y, Huang TT, Carlson E, Kinouchi H, Yang G, Kamii H, Mikawa S, and Kondo T.** Transgenic mice and knockout mutants in the study of oxidative stress in brain injury. *J Neurotrauma* 12: 815–824, 1995.
13. **Chen PY, Gladish RD, and Sanders PW.** Vascular smooth muscle nitric oxide synthase anomalies in Dahl/Rapp salt-sensitive rats. *Hypertension* 31: 918–924, 1998.
14. **Choate JK, Danson EJ, Morris JF, and Paterson DJ.** Peripheral vagal control of heart rate is impaired in neuronal NOS knockout mice. *Am J Physiol Heart Circ Physiol* 281: H2310–H2317, 2001.
15. **Chowdhary S and Townend JN.** Role of nitric oxide in the regulation of cardiovascular autonomic control. *Clin Sci (Lond)* 97: 5–17, 1999.
16. **Cosentino F and Luscher TF.** Endothelial dysfunction in diabetes mellitus. *J Cardiovasc Pharmacol* 32: S54–S61, 1998.
17. **Deng AY and Rapp JP.** Locus for the inducible, but not a constitutive, nitric oxide synthase cosegregates with blood pressure in the Dahl salt-sensitive rat. *J Clin Invest* 95: 2170–2177, 1995.
18. **Ding H, Kubes P, and Triggle C.** Potassium- and acetylcholine-induced vasorelaxation in mice lacking endothelial nitric oxide synthase. *Br J Pharmacol* 129: 1194–1200, 2000.
19. **Duplain H, Burcelin R, Sartori C, Cook S, Egli M, Lepori M, Vollenweider P, Pedrazzini T, Nicod P, Thorens B, and Scherrer U.** Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. *Circulation* 104: 342–345, 2001.
20. **Fagan KA, Tyler RC, Sato K, Fouty BW, Morris KG Jr, Huang PL, McMurtry IF, and Rodman DM.** Relative contributions of endothelial, inducible, and neuronal NOS to tone in the murine pulmonary circulation. *Am J Physiol Lung Cell Mol Physiol* 277: L472–L478, 1999.
21. **Feliciello A, Gottesman ME, and Avvedimento EV.** The biological functions of A-kinase anchor proteins. *J Mol Biol* 308: 99–114, 2001.
22. **Fleming I and Busse R.** NO: the primary EDRF. *J Mol Cell Cardiol* 31: 5–14, 1999.
23. **Godecke A, Decking UK, Ding Z, Hirchenhain J, Bidmon HJ, Godecke S, and Schrader J.** Coronary hemodynamics in endothelial NO synthase knockout mice. *Circ Res* 82: 186–194, 1998.
24. **Godecke A, Heinicke T, Kamkin A, Kiseleva I, Strasser RH, Decking UK, Stumpe T, Isenberg G, and Schrader J.** Inotropic response to β-adrenergic receptor stimulation and anti-adrenergic effect of ACh in endothelial NO synthase-deficient mouse hearts. *J Physiol* 532: 195–204, 2001.
25. **Godecke A and Schrader J.** Adaptive mechanisms of the cardiovascular system in transgenic mice—lessons from eNOS and myoglobin knockout mice. *Basic Res Cardiol* 95: 492–498, 2000.
26. **Guzman NJ, Fang MZ, Tang SS, Ingelfinger JR, and Garg LC.** Autocrine inhibition of $Na^+/K^+$-ATPase by nitric oxide in mouse proximal tubule epithelial cells. *J Clin Invest* 95: 2083–2088, 1995.
27. **Gyurko R, Kuhlencordt P, Fishman MC, and Huang PL.** Modulation of mouse cardiac function in vivo by eNOS and ANP. *Am J Physiol Heart Circ Physiol* 278: H971–H981, 2000.
28. **Han X, Kubota I, Feron O, Opel DJ, Arstall MA, Zhao YY, Huang P, Fishman MC, Michel T, and Kelly RA.** Muscarinic cholinergic regulation of cardiac myocyte ICa-L is absent in mice with targeted disruption of endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 95: 6510–6515, 1998.
29. **Heger J, Godecke A, Flogel U, Merx MW, Molojavyi A, Kuhn-Velten WN, and Schrader J.** Cardiac-specific overexpression of inducible nitric oxide synthase does not result in severe cardiac dysfunction. *Circ Res* 90: 93–99, 2002.
30. **Hogan N, Kardos A, Paterson DJ, and Casadei B.** Effect of exogenous nitric oxide on baroreflex function in humans. *Am J Physiol Heart Circ Physiol* 277: H221–H227, 1999.
31. **Huang A, Sun D, Carroll MA, Jiang H, Smith CJ, Connetta JA, Falck JR, Shesely EG, Koller A, and Kaley G.** EDHF mediates flow-induced dilation in skeletal muscle arterioles of female eNOS-KO mice. *Am J Physiol Heart Circ Physiol* 280: H2462–H2469, 2001.
32. **Huang A, Sun D, Koller A, and Kaley G.** Gender difference in flow-induced dilation and regulation of shear stress: role of estrogen and nitric oxide. *Am J Physiol Regul Integr Comp Physiol* 275: R1571–R1577, 1998.
33. **Huang A, Sun D, Shesely EG, Levee EM, Koller A, and Kaley G.** Neuronal NOS-dependent dilation to flow in coronary arteries of male eNOS-KO mice. *Am J Physiol Heart Circ Physiol* 282: H429–H436, 2002.
34. **Huang PL.** Lessons learned from nitric oxide synthase knockout animals. *Semin Perinatol* 24: 87–90, 2000.
35. **Huang PL.** Mouse models of nitric oxide synthase deficiency. *J Am Soc Nephrol* 11, *Suppl* 16: S120–S123, 2000.
36. **Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, and Fishman MC.** Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature* 377: 239–242, 1995.
37. **Ihrig M, Dangler CA, and Fox JG.** Mice lacking inducible nitric oxide synthase develop spontaneous hypercholesterolaemia and aortic atheromas. *Atherosclerosis* 156: 103–107, 2001.
38. **Ito S, Johnson CS, and Carretero OA.** Modulation of angiotensin II-induced vasoconstriction by endothelium-derived re-



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laxing factor in the isolated microperfused rabbit afferent arteriole. *J Clin Invest* 87: 1656–1663, 1991.
39. **Iyengar R.** Molecular and functional diversity of mammalian Gs-stimulated adenylyl cyclases. *FASEB J* 7: 768–775, 1993.
40. **Jumrussirikul P, Dinerman J, Dawson TM, Dawson VL, Ekelund U, Georgakopoulos D, Schramm LP, Calkins H, Snyder SH, Hare JM, and Berger RD.** Interaction between neuronal nitric oxide synthase and inhibitory G protein activity in heart rate regulation in conscious mice. *J Clin Invest* 102: 1279–1285, 1998.
41. **Kakoki M, Zou AP, and Mattson DL.** The influence of nitric oxide synthase 1 on blood flow and interstitial nitric oxide in the kidney. *Am J Physiol Regul Integr Comp Physiol* 281: R91–R97, 2001.
42. **Kanai AJ, Pearce LL, Clemens PR, Birder LA, VanBibber MM, Choi SY, de Groat WC, and Peterson J.** Identification of a neuronal nitric oxide synthase in isolated cardiac mitochondria using electrochemical detection. *Proc Natl Acad Sci USA* 98: 14126–14131, 2001.
43. **Kohagura K, Endo Y, Ito O, Arima S, Omata K, and Ito S.** Endogenous nitric oxide and epoxyeicosatrienoic acids modulate angiotensin II-induced constriction in the rabbit afferent arteriole. *Acta Physiol Scand* 168: 107–112, 2000.
44. **Kone BC and Baylis C.** Biosynthesis and homeostatic roles of nitric oxide in the normal kidney. *Am J Physiol Renal Physiol* 272: F561–F578, 1997.
45. **Kurihara N, Alfie ME, Sigmon DH, Rhaleb NE, Shesely EG, and Carretero OA.** Role of nNOS in blood pressure regulation in eNOS null mutant mice. *Hypertension* 32: 856–861, 1998.
46. **Kurtz A and Wagner C.** Role of nitric oxide in the control of renin secretion. *Am J Physiol Renal Physiol* 275: F849–F862, 1998.
47. **Lamping KG and Faraci FM.** Role of sex differences and effects of endothelial NO synthase deficiency in responses of carotid arteries to serotonin. *Arterioscler Thromb Vasc Biol* 21: 523–528, 2001.
48. **Li Z, Chapleau MW, Bates JN, Bielefeldt K, Lee HC, and Abboud FM.** Nitric oxide as an autocrine regulator of sodium currents in baroreceptor neurons. *Neuron* 20: 1039–1049, 1998.
49. **Lopez-Farre A, Rodriguez-Feo JA, Sanchez de Miguel L, Rico L, and Casado S.** Role of nitric oxide in the control of apoptosis in the microvasculature. *Int J Biochem Cell Biol* 30: 1095–1106, 1998.
50. **Mashimo H and Goyal RK.** Lessons from genetically engineered animal models. IV. Nitric oxide synthase gene knockout mice. *Am J Physiol Gastrointest Liver Physiol* 277: G745–G750, 1999.
51. **Mattson DL.** Use of antisense techniques in rat renal medulla. *Methods Enzymol* 314: 389–400, 2000.
52. **Mattson DL and Bellehumeur TG.** Neural nitric oxide synthase in the renal medulla and blood pressure regulation. *Hypertension* 28: 297–303, 1996.
53. **Mattson DL, Lu S, and Cowley AW Jr.** Role of nitric oxide in the control of the renal medullary circulation. *Clin Exp Pharmacol Physiol* 24: 587–590, 1997.
54. **Mattson DL, Lu S, Nakanishi K, Papanek PE, and Cowley AW Jr.** Effect of chronic renal medullary nitric oxide inhibition on blood pressure. *Am J Physiol Heart Circ Physiol* 266: H1918–H1926, 1994.
55. **McCulloch AI and Randall MD.** Sex differences in the relative contributions of nitric oxide and EDHF to agonist-stimulated endothelium-dependent relaxations in the rat isolated mesenteric arterial bed. *Br J Pharmacol* 123: 1700–1706, 1998.
56. **Meng W, Ayata C, Waeber C, Huang PL, and Moskowitz MA.** Neuronal NOS-cGMP-dependent ACh-induced relaxation in pial arterioles of endothelial NOS knockout mice. *Am J Physiol Heart Circ Physiol* 274: H411–H415, 1998.
57. **Mori T, Dickhout JG, and Cowley AW Jr.** Vasopressin increases intracellular NO concentration via $Ca^{2+}$ signaling in inner medullary collecting duct. *Hypertension* 39: 465–469, 2002.
58. **Mungrue IN, Gros R, You X, Pirani A, Azad A, Csont T, Schulz R, Butany J, Stewart DJ, and Husain M.** Cardiomyocyte overexpression of iNOS in mice results in peroxynitrite generation, heart block, and sudden death. *J Clin Invest* 109: 735–743, 2002.
59. **Nelson RJ, Demas GE, Huang PL, Fishman MC, Dawson VL, Dawson TM, and Snyder SH.** Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase. *Nature* 378: 383–386, 1995.
60. **Ortiz PA and Garvin JL.** Autocrine effects of nitric oxide on $HCO_3^-$ transport by rat thick ascending limb. *Kidney Int* 58: 2069–2074, 2000.
61. **Ortiz PA and Garvin JL.** NO inhibits NaCl absorption by rat thick ascending limb through activation of cGMP-stimulated phosphodiesterase. *Hypertension* 37: 467–471, 2001.
62. **Ortiz PA and Garvin JL.** Interaction of $O_2^-$ and NO in the thick ascending limb. *Hypertension* 39: 591–596, 2002.
63. **Ortiz PA and Garvin JL.** Role of nitric oxide in the regulation of nephron transport. *Am J Physiol Renal Physiol* 282: F777–F784, 2002.
64. **Ortiz PA, Hong NJ, and Garvin JL.** NO decreases thick ascending limb chloride absorption by reducing $Na^+$-$K^+$-$2Cl^-$ cotransporter activity. *Am J Physiol Renal Physiol* 281: F819–F825, 2001.
65. **Pallone TL and Mattson DL.** Role of nitric oxide in regulation of the renal medulla in normal and hypertensive kidneys. *Curr Opin Nephrol Hypertens* 11: 93–98, 2002.
66. **Patzak A, Mrowka R, Storch E, Hocher B, and Persson PB.** Interaction of angiotensin II and nitric oxide in isolated perfused afferent arterioles of mice. *J Am Soc Nephrol* 12: 1122–1127, 2001.
67. **Paulus WJ and Shah AM.** NO and cardiac diastolic function. *Cardiovasc Res* 43: 595–606, 1999.
68. **Persson AE, Brown R, Liu R, and Ollerstam A.** Nitric oxide modulates and adenosine mediates the tubuloglomerular feedback mechanism. *Acta Physiol Scand* 176: 91–94, 2002.
69. **Plato CF, Shesely EG, and Garvin JL.** eNOS mediates L-arginine-induced inhibition of thick ascending limb chloride flux. *Hypertension* 35: 319–323, 2000.
70. **Plato CF, Stoos BA, Wang D, and Garvin JL.** Endogenous nitric oxide inhibits chloride transport in the thick ascending limb. *Am J Physiol Renal Physiol* 276: F159–F163, 1999.
71. **Ren Y, Arima S, Carretero OA, and Ito S.** Possible role of adenosine in macula densa control of glomerular hemodynamics. *Kidney Int* 61: 169–176, 2002.
72. **Ren Y, Carretero OA, and Garvin JL.** Role of mesangial cells and gap junctions in tubuloglomerular feedback. *Kidney Int* 62: 525–531, 2002.
73. **Ren YL, Garvin JL, and Carretero OA.** Role of macula densa nitric oxide and cGMP in the regulation of tubuloglomerular feedback. *Kidney Int* 58: 2053–2060, 2000.
74. **Ren YL, Garvin JL, Ito S, and Carretero OA.** Role of neuronal nitric oxide synthase in the macula densa. *Kidney Int* 60: 1676–1683, 2001.
75. **Rhinehart KL and Pallone TL.** Nitric oxide generation by isolated descending vasa recta. *Am J Physiol Heart Circ Physiol* 281: H316–H324, 2001.
76. **Rikitake Y, Hirata K, Kawashima S, Ozaki M, Takahashi T, Ogawa W, Inoue N, and Yokoyama M.** Involvement of endothelial nitric oxide in sphingosine-1-phosphate-induced angiogenesis. *Arterioscler Thromb Vasc Biol* 22: 108–114, 2002.
77. **Roczniak A, Zimpelmann J, and Burns KD.** Effect of dietary salt on neuronal nitric oxide synthase in the inner medullary collecting duct. *Am J Physiol Renal Physiol* 275: F46–F54, 1998.
78. **Samdani AF, Dawson TM, and Dawson VL.** Nitric oxide synthase in models of focal ischemia. *Stroke* 28: 1283–1288, 1997.
79. **Scotland RS, Chauhan S, Vallance PJ, and Ahluwalia A.** An endothelium-derived hyperpolarizing factor-like factor moderates myogenic constriction of mesenteric resistance arteries in the absence of endothelial nitric oxide synthase-derived nitric oxide. *Hypertension* 38: 833–839, 2001.
80. **Scotland RS, Morales-Ruiz M, Chen Y, Yu J, Rudic RD, Fulton D, Gratton JP, and Sessa WC.** Functional reconstitution of endothelial nitric oxide synthase reveals the impor-



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tance of serine 1179 in endothelium-dependent vasomotion. *Circ Res* 90: 904–910, 2002.
81. **Shesely EG, Maeda N, Kim HS, Desai KM, Krege JH, Laubach VE, Sherman PA, Sessa WC, and Smithies O.** Elevated blood pressures in mice lacking endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 93: 13176–13181, 1996.
82. **Siegfried G, Amiel C, and Friedlander G.** Inhibition of ecto-5′-nucleotidase by nitric oxide donors. Implications in renal epithelial cells. *J Biol Chem* 271: 4659–4664, 1996.
83. **Skalhegg BS and Tasken K.** Specificity in the cAMP/PKA signaling pathway. Differential expression, regulation, and subcellular localization of subunits of PKA. *Front Biosci* 5: D678-D693, 2000.
84. **Spieker LE, Corti R, Binggeli C, Luscher TF, and Noll G.** Baroreceptor dysfunction induced by nitric oxide synthase inhibition in humans. *J Am Coll Cardiol* 36: 213–218, 2000.
85. **Stauss HM, Godecke A, Mrowka R, Schrader J, and Persson PB.** Enhanced blood pressure variability in eNOS knockout mice. *Hypertension* 33: 1359–1363, 1999.
86. **Stauss HM, Nafz B, Mrowka R, and Persson PB.** Blood pressure control in eNOS knock-out mice: comparison with other species under NO blockade. *Acta Physiol Scand* 168: 155–160, 2000.
87. **Stauss HM and Persson PB.** Role of nitric oxide in buffering short-term blood pressure fluctuations. *News Physiol Sci* 15: 229–233, 2000.
88. **Stoclet JC, Muller B, Gyorgy K, Andriantsiothaina R, and Kleschyov AL.** The inducible nitric oxide synthase in vascular and cardiac tissue. *Eur J Pharmacol* 375: 139–155, 1999.
89. **Stoos BA and Garvin JL.** Actions of nitric oxide on renal epithelial transport. *Clin Exp Pharmacol Physiol* 24: 591–594, 1997.
90. **Stulak JM, Lerman A, Caccitolo JA, Wilson SH, Romero JC, Schaff HV, Porcel MR, and Lerman LO.** Impaired renal vascular endothelial function in vitro in experimental hypercholesterolemia. *Atherosclerosis* 154: 195–201, 2001.
91. **Sun D, Huang A, Smith CJ, Stackpole CJ, Connetta JA, Shesely EG, Koller A, and Kaley G.** Enhanced release of prostaglandins contributes to flow-induced arteriolar dilation in eNOS knockout mice. *Circ Res* 85: 288–293, 1999.
92. **Sun D, Samuelson LC, Yang T, Huang Y, Paliege A, Saunders T, Briggs J, and Schnermann J.** Mediation of tubuloglomerular feedback by adenosine: evidence from mice lacking adenosine 1 receptors. *Proc Natl Acad Sci USA* 98: 9983–9988, 2001.
93. **Takahashi N and Smithies O.** Gene targeting approaches to analyzing hypertension. *J Am Soc Nephrol* 10: 1598–1605, 1999.
94. **Thomson S, Bao D, Deng A, and Vallon V.** Adenosine formed by 5′-nucleotidase mediates tubuloglomerular feedback. *J Clin Invest* 106: 289–298, 2000.
95. **Thorup C, Erik A, and Persson G.** Macula densa derived nitric oxide in regulation of glomerular capillary pressure. *Kidney Int* 49: 430–436, 1996.
96. **Tojo A, Welch WJ, Bremer V, Kimoto M, Kimura K, Omata M, Ogawa T, Vallance P, and Wilcox CS.** Colocalization of demethylating enzymes and NOS and functional effects of methylarginines in rat kidney. *Kidney Int* 52: 1593–1601, 1997.
97. **Ullrich R, Bloch KD, Ichinose F, Steudel W, and Zapol WM.** Hypoxic pulmonary blood flow redistribution and arterial oxygenation in endotoxin-challenged NOS2-deficient mice. *J Clin Invest* 104: 1421–1429, 1999.
98. **Vallon V, Traynor T, Barajas L, Huang YG, Briggs JP, and Schnermann J.** Feedback control of glomerular vascular tone in neuronal nitric oxide synthase knockout mice. *J Am Soc Nephrol* 12: 1599–1606, 2001.
99. **Vandecasteele G, Eschenhagen T, Scholz H, Stein B, Verde I, and Fischmeister R.** Muscarinic and beta-adrenergic regulation of heart rate, force of contraction and calcium current is preserved in mice lacking endothelial nitric oxide synthase. *Nat Med* 5: 331–334, 1999.
100. **Varghese P, Harrison RW, Lofthouse RA, Georgakopoulos D, Berkowitz DE, and Hare JM.** $\beta_3$-Adrenoceptor deficiency blocks nitric oxide-dependent inhibition of myocardial contractility. *J Clin Invest* 106: 697–703, 2000.
101. **Wagner C, Godecke A, Ford M, Schnermann J, Schrader J, and Kurtz A.** Regulation of renin gene expression in kidneys of eNOS- and nNOS-deficient mice. *Pflügers Arch* 439: 567–572, 2000.
102. **Wang H, Carretero OA, and Garvin JL.** Nitric oxide produced by THAL nitric oxide synthase inhibits TGF. *Hypertension* 39: 662–666, 2002.
103. **Wang T.** Role of iNOS and eNOS in modulating proximal tubule transport and acid-base balance. *Am J Physiol Renal Physiol* 283: F658–F662, 2002.
104. **Wang T, Inglis FM, and Kalb RG.** Defective fluid and $HCO_3^-$ absorption in proximal tubule of neuronal nitric oxide synthase-knockout mice. *Am J Physiol Renal Physiol* 279: F518–F524, 2000.
105. **Wang X, Lu M, Gao Y, Papapetropoulos A, Sessa WC, and Wang W.** Neuronal nitric oxide synthase is expressed in principal cell of collecting duct. *Am J Physiol Renal Physiol* 275: F395–F399, 1998.
106. **Wilcox CS.** Role of macula densa NOS in tubuloglomerular feedback. *Curr Opin Nephrol Hypertens* 7: 443–449, 1998.
107. **Wilcox CS and Welch WJ.** Macula densa nitric oxide synthase: expression, regulation, and function. *Kidney Int Suppl* 67: S53–S57, 1998.
108. **Wu XC and Johns EJ.** Nitric oxide modulation of neurally induced proximal tubular fluid reabsorption in the rat. *Hypertension* 39: 790–793, 2002.
109. **Xu KY, Huso DL, Dawson TM, Bredt DS, and Becker LC.** Nitric oxide synthase in cardiac sarcoplasmic reticulum. *Proc Natl Acad Sci USA* 96: 657–662, 1999.
110. **Yang XP, Liu YH, Shesely EG, Bulagannawar M, Liu F, and Carretero OA.** Endothelial nitric oxide gene knockout mice: cardiac phenotypes and the effect of angiotensin-converting enzyme inhibitor on myocardial ischemia/reperfusion injury. *Hypertension* 34: 24–30, 1999.
111. **Zanzinger J.** Role of nitric oxide in the neural control of cardiovascular function. *Cardiovasc Res* 43: 639–649, 1999.



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